ACTION POTENTIAL SHAPE ANALYSIS FOR DETECTING TOXINS IN WARFARE, USING A REALISTIC MATHEMATICAL MODEL OF DIFFERENTIATED NG108-15 CELLS

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ABSTRACT

Currently used warfare agent toxicity detection methods are ideal if the toxic agent is known. For the detection and classification of unknown toxins whole cell biosensors are better suited because they respond to a wider selection of toxic agents at physiological concentrations. The whole cell response to a known or unknown toxin reflects a system-level response that involves multiple components within the cell that function in a coordinated fashion. The utilization of this effect might enable the fabrication of biosensors that detect complex biological phenomena. Now algorithms can be constructed to relate toxin effects to these cellular processes. We are creating whole cell biosensors, which use cultured cells in a defined environment to monitor perturbations in the normal physiological activity of cells caused by an environmental threat or warfare agent.

1. INTRODUCTION

The NG108-15 neuroblastoma / glyoma hybrid cell line is frequently used for toxin detection, pharmaceutical screening and as a whole-cell biosensor. Our new method utilizes action potential shape analysis to relate cellular response to toxin or drug effects. In order to make action potential shape analysis as a toxin sensing method possible, we have created a computer model of the action potential generation of the NG108-15 cells. This model was combined with experimental data to develop an analysis paradigm. Voltage dependent sodium, potassium and high-threshold calcium currents, and also action potentials, were recorded from NG108-15 cells with conventional whole-cell patch-clamp methods.

2. METHODS

Based on classic Hodgkin-Huxley formalism and the linear thermodynamic description of the rate constants, ion-channel parameters were estimated using an automatic fitting method. Using these established parameters, action potentials were generated by the Hodgkin-Huxley model and were fitted to the recorded action potentials. In order to validate action potential analysis as a toxin detection method different toxins were applied (sodium channels: tetrodotoxin, tefluthrin; potassium channels: TEA, 4-AP; calcium channels: nifedipine) and their effect based on the established action potential generation model was analyzed (Fig1). For the approximations of the intracellular action potentials based on extracellular recordings, a mathematical model of the cell-electrode interface was also established.

3. RESULTS

Our experiments indicated that the range of toxins affected the shape of the action potentials differently and their effect could be identified based on the changes in the fitted parameters. The potential application of this whole cell biosensor for defence and military uses could be in areas where an automatic, unattended, remote sensing test system is used in field conditions. It could also be used to detect toxic substances from a variety of sources and to determine the extent of contamination of both personnel and equipment. It could also be used to monitor enclosed environments such as in submarines.

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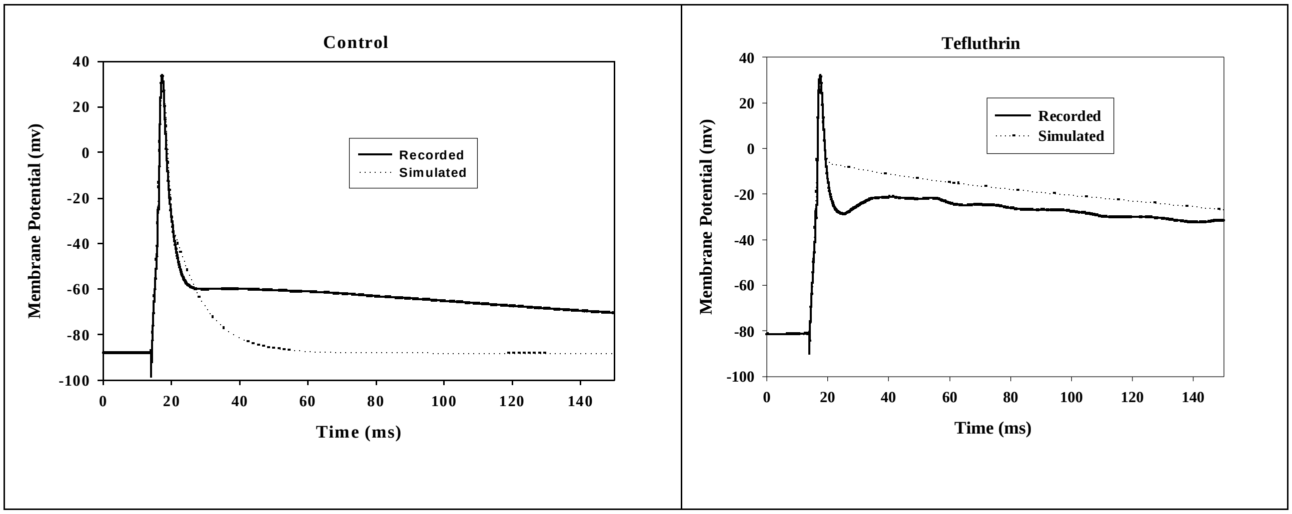

Fig. 1: Effect of a toxin on the action potential of a NG108-15 cell. **Left panel**: Action Potential Control. **Right panel**: Effect of 0.5 μM Tefluthrin.

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CONCLUSION:

Our results represent the first steps to establish a high-throughput toxin detection method based on action potentials recorded from NG108-15 cells with extracellular electrodes. Our mathematical model of the action potential generation and the cell-electrode interface make the quantification and classification of toxin effects possible. Thus new method can also be integrated with other biosensor platforms.